Starch characterization of commercial extruded dry pet foods

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ABSTRACT: Starches provide an effective energy source for dogs and cats and can affect health according to its inclusion and extent of digestion. The starch fraction that escapes small intestine (SI) digestion is called resistant starch (RS) and is desirable due to its prebiotic function. Starch is not an essential nutrient for dogs and cats and thus is not reported on commercial pet food labels. Hence, the objective of this work was to characterize starches in commercial pet foods. The top five pet food companies by sales were selected to represent U.S. pet foods, which were divided into four strata with a sampling frame of 654 foods: dog grain based (372 foods), dog grain free (71 foods), cat grain based (175 foods), and cat grain free (38 foods). Five random foods within each stratum were purchased (20 total). Starch analyses (total starch, resistant starch, and starch cook), as well as nutrient analyses were conducted on all foods. Total starch, RS, and starch cook means were compared using a two-group *Z*-test on dog vs. cat and grain-based (GB) vs. grain-free (GF) diets, and differences were considered significant at a $P < 0.05$. Total starch was higher ($P < 0.05$) in dog than cat food, and starch cook was greater ($P < 0.05$) in GF diets. A regression analysis showed that nitrogen-free extract was a good predictor of total starch. Resistant starch was low and not different among groups. A post hoc test showed that a total sample size of at least 28 diets per group would be required to detect differences in RS between GF and GB diets, if one exists.

Key words: extrusion, gelatinized, grain-free, pet food, resistant starch, starch

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INTRODUCTION

The U.S. pet food industry is a growing market expected to exceed US$30.01 billion by 2022 (Zion Market Research, 2017). Most dogs and cats are fed dry food (US$9.2 billion in sales in 2014; Statista, 2016), and the greatest part of it is produced through extrusion. This type of processing involves cooking with steam, water, and shear. It also requires some amount of structure forming ingredients like starches (Guy, 2001) to promote food particle binding, texturization, improvement in palatability, and to aid in expansion of the kibble.

Starch is not an essential nutrient for dogs and cats, but it can impact health in different ways according to its inclusion, type, and processing. The more cooked or gelatinized, the more rapidly the starch is digested (Murray et al., 2001). This has implications on metabolic utilization, and (or) the amount of starch that escapes digestion. Rapidly digested starches can promote high blood glucose/insulin peaks with subsequent fat deposition (Coulston et al., 1983). Conversely, the indigestible starch, or resistant starch (RS) can serve as substrate for colonic fermentation

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yielding short-chain fatty acids of which butyrate can be used as a direct energy source for colonocytes (Bergman, 1990; Haenen et al., 2013). Starches may be inaccessible to digestive enzymes due to their tightly packed physical conformation, or physical barriers associated with the granule like cell walls and protein bodies (Dhital et al., 2017).

There are differences in the digestion profile among starch sources for a number of reasons. For example, common cereals like corn, rice, or wheat can have polyhedral and (or) oval starch granules which contain pores and channels that create adhesion sites for hydrolytic enzymes (Dhital et al., 2017). Some cereals like sorghum may be more difficult to digest than corn or rice due to tight bonding of protein bodies to the starch granule. Similarly, legume seeds are known to be high in naturally occurring RS, partly because their starches are trapped inside the cotyledon cell parenchyma (Würsch, 1986; Berg et al., 2012). Tuber starches like potato may also have some resistance to enzymatic digestion because its granules are large and smooth (Dhital et al., 2017; Martens et al., 2018). However, all these reports have been conducted with starch ingredients alone, but pet foods are composed of other ingredients which are ground, mixed, and then cooked or processed in some manner. Due to morphological differences in starch ingredients used in pet foods and the interference of other ingredients and processing, it would be valuable to characterize these food starches in a complete food.

There is no information required on pet food labels regarding starch percentage, extent of digestion, and (or) resistant starch concentration. Starch is not required nor allowed on the guaranteed analysis by current labeling regulation (AAFCO, 2019). Typical carbohydrate levels (starches and fibers) in dry extruded dog foods range from 30% to 60%, while starches in commercial cat foods are included up to 35% on a DM basis (Gross et al., 2010). Nutritionists and pet food scientists commonly estimate starch content using the NFE (nitrogen-free extract) calculation (NFE = 100 − moisture − crude fiber − crude protein − crude fat − ash; Gross et al., 2010). This equation may overestimate starch content, as most of the nutrient analysis in pet foods are crude estimates of their true value and may not account for their total contribution. Knowing the true starch content of pet foods, and how much of it is digested, would be valuable information for diet development and future research. No research has been published previously characterizing and comparing the various starch components and methodologies of analysis in commercial complete pet foods. Further, there are no studies comparing the digestible starch and RS of grain-based (GB) foods and those containing elevated levels of tubers and legumes as their sole starch sources. Thus, the objective of this study was to determine the total starch content and its fractions (digestible and resistant starches) in dog and cat foods, and those that are grain-free (GF) and GB diets sold in the United States. The hypotheses were 1) Dog foods would contain more starch than cat diets; 2) Extruded foods would be extensively cooked to a point that resistant starch would be almost nonexistent and thus insufficient to promote colonic health; and 3) GF diets would have more resistant starch in comparison to GB diets.

MATERIALS AND METHODS

Sample Selection

The top five pet food companies by sales (Pet Food Industry, 2019) were selected to represent the majority of U.S. pet foods in this study. These companies, in decreasing order of sales, were Mars Petcare, Nestlé Purina Petcare, Big Heart Pet brands, Hill's Pet Nutrition, and Blue Buffalo (Pet Food Industry, 2019). A list of all dry complete extruded pet foods, excluding prescription diets, of the top five companies was created. Pet foods were divided into four strata with 654 foods composing the sampling frame: dog GB (372 foods), dog GF (71 foods), cat GB (175 foods), and cat GF (38 foods). Four lists with 10 random samples within each stratum were created using a randomization program. These lists were taken to pet stores in Manhattan, KS, and five foods present in the list within each stratum were purchased (20 total), according to store availability (Table 1). Grain-based diets contained combinations of brewers rice (eight foods), brown rice (two foods), corn (five foods), wheat (three foods), barley (four foods), oats (four foods), and some also included peas and potato starch. The GF foods had one ingredient or a combination of some of the following: peas (five foods), pea starch (four foods), sweet potatoes (four foods), potatoes (four foods), potato starch (one food), tapioca starch (three foods), chickpeas (two foods), and lentils (one food).

Nutrient Analysis

All food samples were ground to 0.5 mm in a laboratory fixed blade impact mill (Retsch, type ZM200, Haan, Germany) prior to nutrient analyses.

Table 1. Nutritional composition of the commercial diets used in the study*

Food	Species	Category†	Crude protein	Fat, acid hydrolysis	Ash	TDF	Starch	Moisture	NFE‡
1	Dog	GB	28.8	16.6	7.75	10.34	24.4	6.15	30.4
2	Dog	GB	27.1	10.5	9.20	9.02	32.5	6.82	37.3
3	Dog	GB	29.9	12.9	7.24	6.10	27.9	6.31	37.6
4	Dog	GB	29.2	16.7	7.42	7.43	26.9	5.52	33.7
5	Dog	GB	26.2	15.7	6.66	8.99	30.1	6.22	36.3
6	Dog	GF	23.7	14.3	8.46	9.88	28.3	6.20	37.5
7	Dog	GF	22.5	13.3	6.02	11.46	32.9	6.35	40.3
8	Dog	GF	31.0	16.4	7.83	10.11	22.7	6.30	28.3
9	Dog	GF	22.7	10.7	8.81	8.05	37.5	6.28	43.5
10	Dog	GF	33.2	20.0	7.21	11.83	16.2	5.99	21.7
11	Cat	GB	36.7	17.9	7.03	6.54	23.9	4.04	27.8
12	Cat	GB	31.9	13.0	7.05	10.59	25.8	6.06	31.4
13	Cat	GB	33.3	10.8	7.08	10.20	27.3	4.89	33.7
14	Cat	GB	36.2	11.2	5.86	13.56	19.9	6.07	27.1
15	Cat	GB	36.7	15.2	8.24	10.90	19.4	5.59	23.4
16	Cat	GF	43.8	16.7	7.93	7.92	15.8	4.25	19.4
17	Cat	GF	33.8	11.5	7.04	11.73	22.0	5.81	30.1
18	Cat	GF	37.3	15.5	7.88	7.88	21.1	5.74	25.7
19	Cat	GF	41.4	14.4	7.03	8.18	20.9	4.62	24.4
20	Cat	GF	33.5	14.1	8.23	9.90	22.7	5.63	28.7

*All nutrients reported on a percentage as-is basis.

†GB, grain based; GF, grain-free.

‡NFE was the only calculated component.

Ash (AOAC 942.05), nitrogen (AOAC 990.03; multiplied by 6.25 factor to estimate crude protein), fat by acid hydrolysis (AOAC 954.02), and total dietary fiber (TDF; TDF-100A kit; Megazyme International Ireland Limited, Ireland) were measured on each sample in order to determine NFE, using the following calculation: NFE = 100(%) − ash(%) − moisture(%) − protein(%) − fat(%) − TDF(%). Total starch and starch fractions (resistant and digestible starches) were analyzed with enzymatic digestion followed by colorimetric assays using kits (Total Starch Assay kit & Resistant Starch Assay kit, respectively; Megazyme International Ireland Limited). Total starch was reported as both measured (using the total starch assay kit) and calculated (sum of digestible and resistant starches quantified by the resistant starch assay kit). Starch cook was analyzed by an enzymatic procedure as described by Mason et al. (1982). Briefly, two samples were prepared. One was boiled for 20 min with distilled deionized (DI) water. The second was equilibrated with DI water at 25 °C for 20 min. Then, buffer was added along with glucoamylase enzyme solution to both samples and they were incubated for 70 min at 40 °C. Free glucose in each sample was measured using a biochemistry analyzer (YSI 2900D, Xylem Analytics, Ohio), and the level of gelatinization (%) calculated as a proportion of free glucose in the tested sample (gelatinized) to the free glucose in the boiled sample (total starch).

Statistical Analysis

The study was conducted using stratified random sampling. The averages of each analysis within each stratum were calculated according to Lohr (2009). Treatment means were compared using a two-group *Z*-test with a significance level of $\alpha = 0.05$. A regression analysis was conducted between dietary starch content measured by the total starch procedure (total starch measured) vs. resistant starch procedure (total starch calculated = digestible + resistant starch), and NFE vs. total starch measured, using the PROC REG procedure of Statistical Analysis Software (SAS, v. 9.4; Cary, NC).

RESULTS AND DISCUSSION

The premise of this work was to characterize starch in commercial pet foods to gain some understanding of what is typical regarding starch components to aid further diet development, and to conduct future research in this area. Nutrients were measured in all diets in order to determine NFE, which is a common and rapid method to estimate starch content in diets. As expected, all nutrient

levels met the specified guarantees identified on the label (information not shown). Nitrogen-free extract was calculated using TDF instead of crude fiber, which consists of a more accurate measurement of fibrous components in the food. The regression analysis between total starch measured by the total starch assay kit (Megazyme International Ireland Limited) and NFE ($P < 0.0001$) was:

$$\text{NFE} = 1.04 \times \text{TS} + 3.50$$

The adjusted R^2 and standard error of this regression analysis were 0.94 and 0.0601, respectively. This indicates that NFE correlates well with total starch and thus it is a good estimation of starch content. Likewise, total starch calculated (TS_{calc}) was also highly correlated to total starch measured (TS):

$$TS_{calc} = 0.944 \times TS + 2.08;$$
$$\text{adjusted } R^2 = 0.91, \text{ standard error} = 0.0675$$

The first hypothesis stated that dog foods would contain more total starch than their feline counterparts, due to cats' obligate carnivore nature and higher requirement for protein (NRC, 2006) which would result in a lower starch concentration in their diet. This was confirmed; wherein, total starch measured and calculated in cat diets were lower ($P < 0.05$) than dog diets (Table 2). The total starch (measured) difference between dog and cat foods, with 95% confidence, was estimated to be between 2.26% and 9.87% within the studied sampling frame. When grouping treatments as GB vs. GF, total starch levels were not different.

Table 2. Total, digestible, and resistant starches of dog vs. cat diets, and grain-based (GB) vs. grain-free (GF) diets

Item, %	Dog $n = 10$	Cat $n = 10$	SEM	T	P
Total starch, measured	30.1	24.0	1.94	3.1218	0.0018
Total starch, calculated	31.4	25.1	2.16	2.9122	0.0036
Resistant starch*	0.945	0.703	0.2212	1.0950	0.2735
Digestible starch*	99.0	99.3	0.22	1.1418	0.2535
Starch cook†	88.3	89.2	2.51	0.3498	0.7265
Item, %	GB $n = 10$	GF $n = 10$	SEM	T	P
Total starch, measured	28.4	26.7	1.14	0.6361	0.5247
Total starch, calculated	29.9	26.4	1.52	1.6016	0.1092
Resistant starch*	0.828	1.062	0.1602	0.6360	0.5248
Digestible starch*	99.2	98.9	0.16	0.9158	0.3598
Starch cook†	87.5	94.1	1.46	3.9030	<0.0001

*Resistant and digestible starches were calculated as percentages of the total starch.

†Starch cook calculations were based on total starch and starch gelatinized measured at a commercial laboratory (Wenger Technical Center; Wenger Mfg., Sabetha, KS).

In the present study, we found that commercial diets averaged above 87.5% starch cook, and there was a difference ($P < 0.05$) in starch cook between GB and GF diets (87.5% vs. 94.1%, respectively). Tubers compose a large fraction of GF diets, and it was expected that tuber starches would have a greater degree of cook, since they have a higher water solubility index (Mishra and Rai, 2006; Nuwamanya et al., 2011) and gelatinize at a lower temperature than cereals (Mishra and Rai, 2006). Pezzali and Aldrich (2019) found that a GF dog food with a blend of tapioca starch, potato, and peas required lower extruder thermal energy to produce kibbles with similar bulk density when compared with an ancient grain diet (composed of spelt, millet, and sorghum), and the degree of starch cook of the GF treatment was also high and comparable to the present study (96.8% vs. 94.1%, respectively).

An important premise of this study was to determine the average level of RS in commercial diets. In order to compare starch fractions of different formula diets, digestible and resistant starches were calculated as a percentage of the total starch, so they would be on the same basis. The second hypothesis stated that commercial extruded diets would be low in RS, and indeed the RS levels of all commercial diets were observed to be less than 1% of the starch content (0.945% vs. 0.703% resistant starch in dog vs. cat diets, respectively; Table 1). This may not be sufficient RS to promote colonic health. Peixoto et al. (2018) were able to detect positive differences in colonic fermentation with 1.46% RS as a percentage of total kibble weight, which increased butyrate production and improved nutrient absorption. Another beneficial effect from resistant starch is the reduction of the glycemic index of the food (Kimura, 2013), which decreases the rate of insulin release and positively impacts health. This can help reduce the incidence of obesity and type 2 diabetes.

The third hypothesis was that GF would have more RS than GB diets. Tubers and legumes are common ingredients in GF diets, and they are known to have some resistance to α-amylase digestion due in part to low or absent starch granule pores, while most cereal starches have pores and channels that increase surface area for enzyme adsorption (Dhital et al., 2017; Martens et al., 2018). Also, most legumes have a protein matrix tightly bonded with starch granules, which

create a physical barrier to enzymatic digestion (Berg et al., 2012; Dhital et al., 2017). Moreover, type C starch present in legumes has a lower swelling capacity than cereals or tubers (Wani et al., 2016), and a higher amylose content (Martens et al., 2018), which contribute to enzymatic resistance. Hence, one would expect GF diets to be higher in RS than a GB recipe. However, in the present study there was no difference ($P > 0.05$) in RS between GB and GF diets (0.83% vs. 1.06%, respectively). Although RS content was numerically greater in GF diets, the analytical technique employed has a high degree of variation when RS levels are below 2%. This high variability could influence the ability to detect differences. A retrospective power analysis (post hoc) using RS as the endpoint of GF vs. GB diets, showed that statistical analysis of the present study (using 10 diets as sample size) resulted in a power of only 0.52. This means that the study had a 52% probability to correctly lead to rejection of a false null hypothesis (RS in GB = RS in GF diets). In order to obtain a power of 0.80, with a significance level of 0.05, it would require 28 observations per treatment to detect some difference, if one exists. It is important to note that a statistical difference in dietary RS does not necessarily mean it would have biological significance in the animal.

CONCLUSION

In this study all the commercial diets tested had a very low RS level (close to or less than 1% of the total starch), which is less than what would be considered sufficient to promote colonic health. The level of starch cook did not reflect the amount of RS in the foods, possibly due to the analytical procedures themselves. When grouping treatments as GF vs. GB diets, there was no difference in total starch and RS levels, whereas a difference in total starch content between dog and cat foods was observed. Another important conclusion from this work was that regression analysis of NFE and total starch (calculated) showed that these were good predictors of total starch measured. Although an expanded and uniform kibble is aesthetically pleasing, a less expanded, denser kibble with less gelatinized starch might yield more RS. This work would suggest that different processing considerations than currently used in commercial products would be necessary to shift starch toward greater RS and thereby benefit colonic health.

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